SPERMATOGENESIS OF THE MYRIOPODS.

VI. An Analysis of the Chromosome Group of *Scolopenda heros*.[1]

M. W. BLACKMAN.

During the last few years a number of attempts have been made to analyze the chromosome complex of various animals. These attempts have met with such apparent success in the case of several insects, notably Orthoptera, that I have been led to attempt a similar analysis of the chromosome group in *Scolopendra heros*. Indeed, before the appearance of earlier papers upon this species such an attempt had been made, but it had met with but small success owing, as I now know, to deficiencies in the optical apparatus employed. With the facilities then at my disposal, it was impossible to secure definite clear-cut images of the chromosomes at a magnification greater than 1,500 diameters. As the chromosomes in *Scolopendra heros*, although exceedingly clear-cut and definite in outline, are considerably smaller than in some insects, a greater magnification than 1,500 diameters is necessary if the study is to be at all convincing, either to the investigator or to those reading his report. However, by the use of a Zeiss 2-mm. apochromatic objective and a number 12 compensating ocular, the source of light being a Welsbach mantle, a magnification of 2,300 diameters was obtained with no perceptible loss of definition in the image.

The material used in this study is the same which served as a basis of several previous papers[2] (Blackman :01, :03, :05), the

[1]From the Zoölogical Laboratory, Syracuse University, Syracuse, New York.

[2]Blackman, M. W., :01, "The Spermatogenesis of the Myriapods—I., Notes on the Spermatocytes and Spermatids of *Scolopendra*," *Kans. Univ. Quart.*, Vol. 10, pp. 61–76, pl. 5–7.

Blackman, M. W., :03, "The Spermatogenesis of the Myriapods—II., On the Chromatin in the Spermatocytes of *Scolopendra heros*," BIOL. BULL., Vol. 5, pp. 187–217, 22 fig.

Blackman, M. W., :05, "The Spermatogenesis of the Myriapods—III., The Spermatogenesis of *Scolopendra heros*," *Bull. Mus. Comp. Zoöl., Harvard Coll.*, Vol. 48, No. 1., pp. 1–137, 9 pl.

majority of the slides having been mounted nine years, but the stain (Heidenhain's iron-alum hæmatoxylin), except where a portion of the section extends from under the cover-glass, is as perfect as when first mounted.

Sutton,[1] :02, Robertson,[2] :08, and Nowlin,[3] :08, working upon the male cells of Orthoptera have found that the chromosomes during both the spermatogonial and spermatocyte generations may be arranged in a graded series as regards size. In the spermatogonia this series is a double one, the two chromosomes of a given size representing the similar elements derived from the two parents. These similar chromosomes unite during synapsis (Sutton, :02, *op. cit.*), and give rise to the single series characteristic of the spermatocytes. The extreme difference in the size of the chromosomes in the Orthoptera is so marked that it is noticeable at a glance (see Sutton, :02, Fig. 6) and after studying preparations of this material one cannot doubt the accuracy of their observations or deny the strength of their conclusions, that in these forms the chromosomes at any given stage bear a certain size relation to each other, and that this presents strong evidence in support of the theory of the individuality of the chromosomes.

But if we grant these conclusions with regard to the forms studied, does it necessarily follow that these conclusions should be made more general and applied to the chromosomes of all animals? What shall we say as regards the application of this test to the chromosomes of a form in which the difference in size is not so marked or in which the chromosomes all appear of nearly one size? Such is apparently the case in *Scolopendra*. At ordinary magnification there is very little difference in the size of the chromosomes as seen in a metaphase of the first spermatocyte. *Some* difference is to be detected even at a magnification as low as 1,000 diameters, but this is so slight that if size alone be used as a criterion it would seem impossible to distinguish between the chromosomes farther than to say: "This is one of the smaller ones" or "one of the larger ones."

[1]Sutton, W. S., :02, "On the Morphology of the Chromosome group in *Brachystola magna*," BIOL. BULL., Vol. 4, pp. 24–39, 11 fig.

[2]Robertson, W. R. B., :08, "The Chromosome Complex of *Syrbula admirabilis*," *Kansas Univ. Sci. Bull.*, Vol. IV., pp. 275–305, 5 plates.

[3]Nowlin, Nadine, :08, "The Chromosome Complex of *Melanoplus bivittatus* Say," *Kans. Univ. Sci. Bull.*, Vol. IV., pp. 265–271, 2 plates.

But other tests may be applied and have been applied. Baumgartner[1] (:04), made an attempt to distinguish the chromosomes in *Gryllus* by differences in form. He reaches the conclusion that in *Gryllus* certain definite shapes constantly occur and establishes the probability that there is a fixed number of each type. Davis[2] (:08), working upon various Orthoptera reaches the conclusion that "In addition to the difference in volume, the bivalent autosomes (chromosomes) show constant and characteristic differences in form. In general several more or less distinct morphological types can be distinguished, and the members of each type appear to bear a constant numerical relationship to each other."

Robertson, :08 (*op. cit.*), does not consider the shape of the chromosome of first importance in establishing its identity but considers size as the primary characteristic, while shape is secondary and to a certain extent dependent upon size or at least upon the degree of lengthening. The main criticism I wish to make regarding Robertson's conclusions on this point is that in his study of the chromosomes, he has not drawn them from the best view point to establish any characteristic difference in shape. His drawings are all or nearly all of chromosomes as seen in polar view, whereas a view at right angles to the spindle is more satisfactory in determining both the shape of the chromosomes and their relation to the mantle fibers.

In *Scolopendra*, as I have already implied, it is impossible to establish the identity of many of the chromosomes on the basis of size alone. Early in my work, however, after six or eight chromosome groups had been carefully drawn, it became evident that the chromosomes as seen in a lateral view of the metaphase of the first spermatocyte are of several distinct types as regards shape and that the size relation of the chromosomes of each type are such as to make it possible to distinguish the individual chromosomes with some degree of certainty. This, I think, will be apparent from a study of the figures of plates I. and II., although it must be borne in mind that the figures are of course much less satisfactory for this comparison than the actual chromosomes,

[1]Baumgartner, W. J., :04, "Some New Evidences for the Individuality of the Chromosomes," BIOL. BULL., Vol. 8, pp. 1–23, 3 plates.

[2]Davis, H. S., :08, "Spermatogenesis in Acrididæ and Locustidæ," *Bull. Mus. Comp. Zoöl. Harvard Coll.*, Vol. LIII., pp. 59–158, 9 plates.

due to the fact that many of the chromosomes do not lie at right angles to the line of vision and must, therefore, appear foreshortened in an outline drawing.

Before discussing the individual characteristics of the various chromosomes as seen in metaphase, it might be well to give a brief review of their history in the prophase. The spermatocyte chromosomes are seventeen in number. Of these, sixteen are bivalent elements formed by the end to end union of univalent chromosomes during the tetophase of the last spermatogonial division (Blackman, :03, :05, *op. cit.*). The seventeenth element, the accessory chromosome, is univalent in character, being derived directly from a single specialized chromosome, the accessory chromosome of the spermatogonium. The growth period following synapsis is of long duration in *Scolopendra*, and during this period all of the chromosomes are grouped together to form a nucleolus-like structure to which I have given the name karyosphere. While in the karyosphere the chromosomes are so closely aggregated that their individual outlines cannot be distinguished with certainty. They, however, enter the karyosphere as distinct individuals and later arise from it as definite chromatin segments, similar in every respect except for their greater size. These facts would seem to argue for, rather than against, the individuality of the chromosomes during this stage.

In the prophase the chromosomes arising from the karyosphere are typically long, slender threads of granular chromatin, which invariably show near their middle an interruption of the chromatin—this representing the point at which the chromosomes united during synapsis. The two spermatocyte divisions always result in a longitudinal and a cross division of these bivalent elements. The longitudinal division as a rule seems to occur first, although, as we shall see later, this is not invariable, even for the ordinary chromosomes. The cross division or reduction division results in the separation of entire spermatogonical chromosomes, the division occurring at the point at which they united during synapsis. However, although the results of these divisions are the same for all of the chromosomes (with the exceptions to be noted later), the changes through which the tetrad pass in the prophase and the shapes they assume during the pro-

phase and later during the metaphase differ to some extent. As this difference in shape is one characteristic by which we must hope to identify the various chromosomes, occasion may be taken here to describe briefly the processes by which these various forms arise.

What I shall call type A is represented in the text-figure I. The origin and evolution of this type of tetrad is described in sufficient detail in previous papers (Blackman, :03, :05, *op. cit.*); so it will not be necessary to repeat the description in detail.

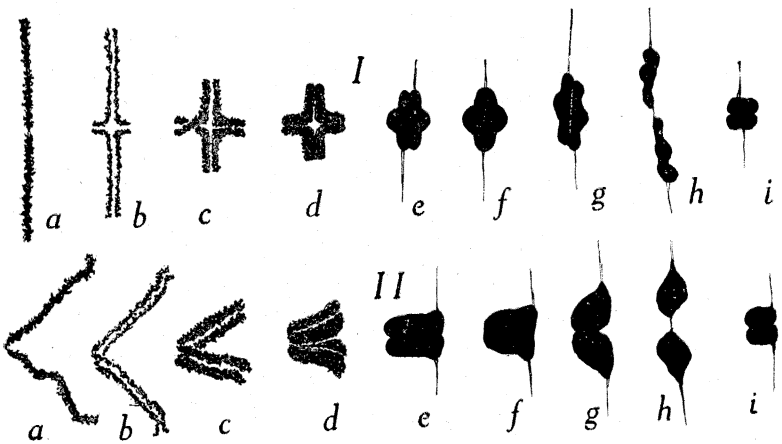

FIG. I. Semi-diagrammatic representation showing the formation and history of the cross-shaped type of tetrads; *a*, bivalent chromatin segment as it appears in the very early prophase; *b*, planes of longitudinal and transverse cleavage established; *c*, *d*, later stage in evolution of prophase tetrad; *e*, *f*, tetrads as seen in early metaphase; *g*, tetrad in act of division, showing the manner in which the component parts glide over each other; *h*, early anaphase showing distortion of halves of tetrad due to their close adhesion; *i*, daughter chromosome in metaphase of second spermatocyte.

FIG. II. Corresponding stages in evolution of the double-V type of tetrad.

I wish, however, to emphasize two points. First the points at the ends of the shorter arms of the cross-like figure (Fig. I, *b*, *c*, *d*) represents the point at which union occurred during synapsis. The attachment of the mantle fibers in the metaphase is *not at this point* as it is said to be in *Syrbula* by Robertson (:08, *op. cit.*), but is at the ends of the longer arms of the cross as shown in Fig. I, *e*, *f*, *g*.

Robertson believes that the attachment of the mantle fibers

coincides with the point of synaptic union of the elements and that each bivalent chromosome during its division undergoes a "change of its long axis from a longitudinal to a transverse direction." This is accomplished by a rotation of the chromatids over each other in such a manner as to result in a longitudinal division of the tetrad in the first spermatocyte. In *Scolopendra*, as I have shown in previous papers (*op. cit.*), no such complicated process occurs in division. The long axis of the tetrad in most cases remains parallel to the line of longitudinal cleavage and in the metaphase the two halves glide over each other during the act of division. As may be seen in the semi-diagrammatic drawings (Fig. I, *g*, *h*) and in several of the chromosomes of this type in the accompanying plates, the two halves of the tetrad seem to adhere rather closely and there is often considerable distortion. In Fig. I, *h*, drawn from my preparations direct it will be seen that the parts of the two daughter chromosomes remaining longest in contact are considerably lengthened and distorted apparently due to the firm adhesion of the two parts.

The second type of spermatocyte chromosome is the "double-V" tetrad described by me in a previous paper (*op. cit.*). This type usually arises from the bivalent chromosomes of the early prophase which are bent at a sharp angle at the point of synapsis. After the longitudinal cleavage of the chromatin thread has occurred the double thread becomes shorter and thicker, resulting in the double-V-shaped structure shown in Fig. II, *c*. There is at all times a very apparent interruption of the chromatin at the angle of each thread (point of synapsis), and it is at this point that the cross division occurs later. In the late prophase there is a still further condensation of the chromatin and shortening of the thread, resulting in the closer apposition of the ends of the threads farthest from the point of synapsis, resulting in a chromosome of the shape shown in Fig. II, *e*, *f*. At the time of the formation of the spindle the mantle fibers come to be attached to the distal ends (ends farthest from point of synapsis) of the tetrad in such a manner (Fig. II, *e*, *f*, *g*) that the chromosome is divided along its longitudinal axis. In this type also, the two halves of the chromosome seem to adhere closely and to divide reluctantly (Fig. II, *g*, *h*, also Figs. 6, *i*, and 17, *j*).

The chromosomes of the third type arise from thread-like structures similar to those from which type A and B arise. This thread may be either approximately straight, or it may be curved slightly in various ways, but is never bent at a sharp angle at the point of synapsis. The filament undergoes a longitudinal cleavage just as with the other types. The two resulting threads, as a usual thing, lie parallel to each other (Fig. III, *b*, *c*) but in some

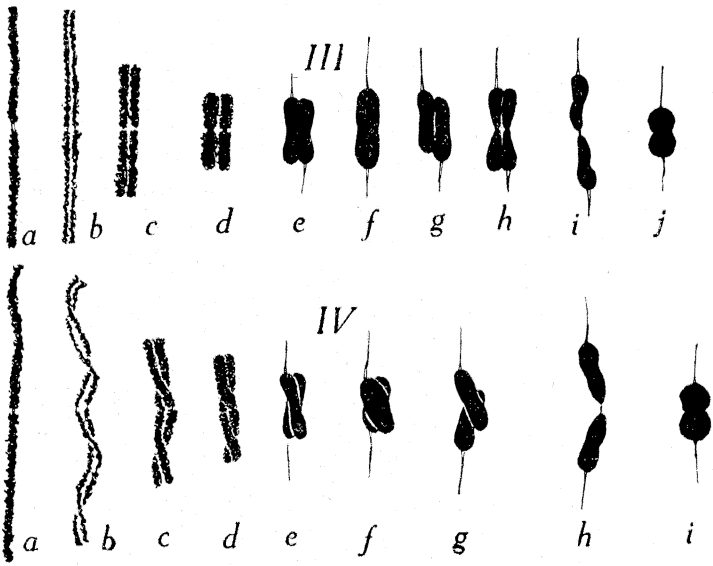

FIG. III. Evolution of the double-rod-shaped tetrads. *a*, bivalent chromatin segment; *b*, *c*, *d*, formation of tetrad; *e*, *f*, tetrads as seen in early stages of the spindle; *g*, *i*, ordinary tetrad in two stages of longitudinal division; *h*, rod-shaped tetrad apparently in act of transverse division; *j*, dyad as seen in metaphase (secondary spermatocyte).

FIG. IV. Variation of double-rod-shaped tetrad. In early prophase the double chromatin segment is often twisted as shown in *b*. The shortening of chromatin thread results in less and less twisting; so that the two parts of the metaphase chromosome merely overlie each other at an angle or are only partially wrapped about each other.

cases they are twisted about each other, so as to form a rope-like structure (Fig. IV, *b*, *c*). In such cases the resulting chromosome has a somewhat different shape. In this type of chromosome the tetrad resulting appears rod-shaped or double rod-shaped depending upon the angle from which the structure is viewed.

After the planes of longitudinal and cross division are established, the further changes involve merely a shortening and condensation of the entire structure. In Fig. III, *c*, *d*, two stages in this process are shown, both cleavages being very evident. Later, as the condensation continues these planes of cleavage are obscured to some extent and usually the only evidence we have of the longitudinal cleavage is a very definite notch at each end of the chromosome, while the plane of cross division is usually shown merely by a slightly lessened diameter of the tetrad near its middle (Fig. III, *d*, *e*, *f*). In some cases, however (Fig. 3, *g*, *h*), the planes of longitudinal and transverse cleavage may be seen very distinctly even in the metaphase (Fig. 4, N, 13, *n*, 16, *n*, etc.). In metakinesis a longitudinal division is accomplished by a gliding apart of the two halves of the tetrad (Fig. III, *g*, *i*) in a manner essentially similar to the division of the cruciform tetrads.

In a number of the cells studied there seems to be one of the chromosomes of this type, which presents quite a different appearance during the act of division. A constriction appears in this chromosome at about the middle point (the plane of transverse cleavage). This is so pronounced that there is in many cases (dependent on the stage of division) a partial or even a complete interruption of the chromatic material at this point (Figs. 3, N, 4, N, 9, N, 10, N, etc.). From a careful study of this chromosome in many cells, the conclusion seems inevitable that this one tetrad undergoes a transverse division, while the rest of the chromosomes are dividing longitudinally.

A variation of the rod-shaped tetrad is shown in text-fig. IV. Here after the longitudinal cleavage has been established, the two threads, instead of lying side by side, are twisted about each other in such a way as to form a rope-like structure. The resulting metaphase chromosome differs somewhat in appearance from others of this type, although in all essentials it is identical. As the threads shorten the twisting gradually becomes less and less pronounced (Fig. IV, *b*, *c*, *d*, *e*) until in the completed chromosomes there is only a slight twisting and usually the two parts merely overlie each other at an angle. The division accomplished by the first maturation mitosis is a longitudinal one.

Besides these three types of ordinary chromosomes all of which

are bivalent there is one element which is univalent in character, being derived directly from a single spermatogonial chromosome. This is the accessory chromosome and in the metaphase can always be distinguished by its characteristic shape, and especially by the fact that it is connected by mantle fibers to only one pole of the spindle. There is usually no indication of the plane of division in the accessory chromosomes at this time, although in the prophase a longitudinal constriction is often shown.

The chromosomes of *Scolopendra heros* are seventeen in number, sixteen of which are bivalent, while one is univalent. The sixteen bivalent chromosomes undergo longitudinal and transverse divisions during the prophase and at the beginning of the metaphase are of several different types as regards shape. After studying a large number of metaphases of both the large and small (Blackman, :05) type of spermatocytes it has been found to be a rule that in each cell the chromosomes of the several types bear a definite and constant numerical relationship to each other. This fact can best be appreciated by referring to the accompanying plates. It will be seen at a glance that the cruciform tetrads which have been described above as type A are in all cases six in number. Furthermore, among the chromosomes of this typical form a definite size relation exists which makes it possible to arrange the cross-shaped chromosomes in a graded series on the basis of bulk. To be sure, the difference in size is not so striking as that existing between the largest and smallest chromosomes in some insect material, but I believe is as great as the difference in size between adjacent chromosomes of the graded series in insects. It is perhaps unnecessary to explain that the *actual* size difference is in many cases greater than appears in the camera lucida drawings, owing to the fact that some of the chromosomes are foreshortened on account of the angle from which they are viewed. For this reason, the drawings are much less convincing than the preparations.

The shape of the chromosomes, aside from showing an apparent modification due to the angle of vision, actually does vary considerably but each of the group of six chromosomes in question are always reducible to the cruciform tetrad described as type A. The variations in shape have to do only with the degree to which

the short arm of the cross is drawn out and to apparent differences incident to the angle of vision. When the short arm of the cross is drawn out but little the tetrad approaches the rod-shaped chromosomes of type C. Then, too, the cruciform tetrads vary in shape in different stages of the metaphase. The limbs of the cross are more likely to be of nearly equal size in the early spindle stages than when division has actually begun. As the chromosomes even in the same equatorial plate, are not all in exactly the same stage of division at the same time this factor should receive consideration.

An attempt was made to learn whether any of the chromosomes constantly either lag behind or precede the others in division. It was found that chromosome A, the largest of the cross-shaped tetrads, shows a tendency to lag somewhat behind the others of this type. In many of the chromosome groups this is very evident. In some cells it is not yet oriented in the characteristic manner in the equatorial plate at a time when some of the others have begun to show the characteristic change in shape incident to the gliding apart of their component elements. This can be seen by comparing chromosome A with other chromosomes of similar type in Figs. 1, 3, 4, etc. Then, too, this chromosome often presents a less clear-cut outline than do the others, approaching the granular condition characteristic of the prophase.

Of the chromosomes of type B there are five present in the metaphase of *Scolopendra heros*. This is the type of chromosome which shows the characteristic double-V shape in the prophase. The difference in shape between the tetrads of this type and the cross-shaped elements is usually quite striking. Even more characteristic of this type is the attachment of the mantle fibers and the orientation of the chromosomes in the equatorial plate. As seen in side view, the chromosome is more or less rectangular in shape, but one end is usually wider than the other and to the angles of this end the mantle fibers are attached. The chromosome usually lies with this end toward the center of the spindle, while the free end (that to which the mantle fibers do not attach) extends outward. This free end is often notched and this notch indicates the plane of longitudinal division. In end view (Figs. 1, *I*, 6, *J*, etc.) the appearance is not so characteristic in the draw-

ings, although in the preparations there is no difficulty in recognizing the true shape of the chromosome, the apparent difference in shape being due to the view-point from which it is seen.

The five chromosomes of type B form a graded series as regards size, just as with those of type A. The largest one is very perceptibly greater than the smallest, and the intermediate ones differ in size to such an extent that there is usually little difficulty in assigning them to their proper place in the series. No individual of this type shows any constant precocity or tardiness in division, although in some cells one or more of them are farther along than the others (Figs. 6, *I*, 17, *J*).

The rod-shaped tetrads (type C) are five in number in *Scolopendra heros*. They show the same constancy in size relation as do the other types, and may be readily arranged in a graded series. Usually one or more of this type of chromosome show the component parts overlapping each other at an angle or partially wrapped around each other, indicating that they arise from the twisted threads often seen in the prophase and already described. However, these are not constant in occurrence and this condition seems to depend largely upon chance.

A fact which has proved rather puzzling was brought out by a careful study of the various chromosomes of this type. While it cannot be doubted that four of the rod-shaped chromosomes divide longitudinally in the first spermatocyte division, the fifth tetrad of this shape apparently divides transversely. In all cases in which this element is well advanced in the metaphase there is a very evident constriction at its middle point, and in some cases this amounts to a nearly complete interruption of the chromatic material. This is especially evident in Figs. 3, 4, 10, chromosome *N*. Indeed, it seems hardly possible to escape the conclusion that at the same time the other fifteen bivalent chromosomes are undergoing longitudinal division, this one element is divided reductionally.

This, however, is no less to be expected than is the behavior of the accessory chromosome in this same division. It differs from the other chromosomes in being univalent (*i. e.*, it has no synaptic mate), while the rest are bivalent. After the formation of the spindle it lies among the other chromosomes and is scarcely distinguishable from the rod-shaped ones aside from the fact that

it is connected by mantle fibers to only one pole of the spindle. It is not divided by the first spermatocyte division but passes to one pole entire. Thus the result is in a sense similar to a reductional division, the two cells differing as regards the distribution of this element. It has no synaptic mate and is, therefore, distributed to only one of the resulting cells (Figs. 19, 20).

It is evident that the unequal distribution of the accessory chromosomes produces two sorts of secondary spermatocytes—one half having only the sixteen ordinary chromosomes and one half having the accessory chromosome in addition. By the second spermatocyte division, the accessory chromosome is divided and as it occurs in but half of the second spermatocytes it is distributed to only half of the spermatids, thus giving rise to a dimorphism among the spermatids and spermatozoa. The significance of this dimorphism has been discussed by a number of investigators—McClung[1] (:02), Wilson[2] (:06), Stevens[3] (:08, :08*a*, :09) Boring[4] (:07) and others—and, as I have nothing new in the way of observations to offer it would appear hardly profitable to consider the subject in detail. I believe, however, that when the chromosomes of the female germ cells of *Scolopendra* are studied it will be found that these are thirty-four in number in the ovogonia, and that the following fertilization formulæ of Wilson (:06, *op. cit.*) will hold good for this species:

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} \begin{pmatrix} \text{including} \\ \text{accessory} \end{pmatrix} = N \text{ (female).}$$

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} - 1 \begin{pmatrix} \text{accessory} \\ \text{lacking} \end{pmatrix} = N - 1 \text{ (male).}$$

[1]McClung, C. E., :02, "The Accessory Chromosome—Sex Determinant?" BIOL. BULL., Vol. 3, pp. 43–84.

[2]Wilson, E. B., :06, "Studies on Chromosomes—III., The Sexual Differences of the Chromosome Group in Hemiptera, with some Considerations on the Determination and Inheritance of Sex," *Journ. Exp. Zoöl.*, Vol. III., pp. 1–40, 6 fig.

[3]Stevens, N. M., :08, "A Study of the Germ Cells of Certain Diptera with Reference to the Heterochromosomes and the Phenomena of Synapsis," *Journ. Exper. Zoöl.*, Vol. V., pp. 359–374, 4 plates.

Stevens, N. M., :08*a*, "The Chromosomes in *Diabrotica vittata*, etc.," *Journ. Exper. Zoöl.*, Vol. V., pp. 453–469, 3 plates.

Stevens, N. M., :09, "Further studies on the Chromosomes of Coleoptera," *Journ. Exper. Zoöl.*, Vol. VI., pp. 101–113. 4 plates.

[4]Boring, Alice M., :07, "A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ," *Journ. Exp. Zoöl.*, Vol. IV., pp. 469–512, 9 plates.

In *Scolopendra* (Blackman, :05, *op. cit.*) there are two distinct types of spermatocytes readily divisible on the basis of size. Those characterized by the larger size are about twice the average diameter of the smaller ones and vary from them in behavior in the two maturation divisions. But this variation in behavior concerns the achromatic structures of the cell and seems to be due to the much greater amount of cytoplasmic and archoplasmic material present in the larger cells. It is extremely interesting to note that as regards the behavior of the chromosomes these two sizes of spermatocytes are essentially identical. Indeed, the chromosome groups of the small cells differ in no respect from those of the large type. The elements are no smaller than in many of the large cells and present the same constancy in form and the same size relations characteristic of the large type of cells. Figs. 16, 17, 18 represent the chromosome groups of the small type of spermatocytes. Many more were carefully studied and drawn and all show the same characteristic shapes and size relations typical of the spermatocyte chromosomes. In fact, the only reason more of these were not used is that they are not so desirable for study owing to the difficulty in drawing them due to their closer crowding in the metaphase.

The shape of the daughter chromosomes as they move apart to the poles in the anaphase of the first spermatocyte mitosis is quite characteristically different for the different types of chromosomes (Figs. I, II, IV, *h*, III, *i*). Those resulting from the division of cross-shaped tetrads have the three lobed appearance shown in Fig. I, *h*. The daughter chromosomes resulting from the division of the double-V-shaped tetrads have the shape shown in Fig. II, *h*, and are essentially single-V-shaped chromosomes, as is shown at a later stage. Those resulting from the division of the double-rod tetrads, as they move toward the poles, have the form of single rods, slightly constricted near the middle. I have been unable to identify positively the division products of the tetrad which undergoes its reduction division in the first mitosis, but in several anaphases six of the daughter chromosomes of each group are V-shaped, and it is probable that the sixth one of this shape is the chromosome in question. This is rendered more certain by the observation that in these cells there are but

four of the rod-shaped chromosomes exclusive of the accessory, which, of course, is present in but one of the chromosome groups.

The chromosome groups, as seen in the metaphase of the two cells derived from one primary spermatocyte are shown in Plate II., Figs. 19, 20. The most striking fact to be observed is the absence of the accessory chromosome in one of the cells. Even a superficial examination, however, shows that in shape and relation to the mantle fibers the chromosomes are of several different types, and that these characteristics coincide with what would be expected from a study of their earlier history. The chromosomes derived from the cross-shaped tetrads have altered their shape considerably since last seen in the anaphase of the first maturation division. They are much shorter and thicker and are now bilobed bodies—the constriction between the lobes representing the plane of transverse cleavage. The attachment of the mantle fibers seems to be at no particular point but may be at any part of each flattened end of the dyad.

The structural peculiarities of the chromosomes derived from the double-V-shaped tetrads are much more characteristic. In shape the dyads of this type resemble those just described to some extent, but, except in size, bear a more striking resemblance to the double-V-shaped chromosomes from which they are derived. One end, usually the one nearest the center of the equatorial plate, is broader than the other, and the entire structure very evidently corresponds to the single-V-shaped chromosomes of the first spermatocyte anaphase. The mantle fibers are always attached to the broader end of the dyad, this fact being even more characteristic than the shape. The appearance of these chromosomes while in the act of division might lead one to believe that the resulting division is a longitudinal one, but such a conclusion would ignore entirely the previous history of this type of chromosomes during the prophase and metaphase of the first maturation division.

Chromosomes L, M, O and P, in the second spermatocyte (Figs. 19, 20) are the product of the longitudinal division of the rod-shaped tetrads. They are dumbbell-shaped dyads with a mantle fiber attached to each end. The constriction at the middle of each represents the plane of transverse division. Chro-

mosome N shows a considerably different shape, corresponding to its different history. It is shorter and in one plane broader than the other dyads derived from the double-rod-shaped tetrads of the first spermatocyte. It has been already shown that chromosome N of the first spermatocyte probably undergoes a transverse division while the other tetrads are dividing longitudinally, and we would, therefore, expect the products of this division to present a different appearance from the other dyads. As a matter of fact, it is of quite a different shape from the others derived from the double-rod tetrads.

The differences in size between the various chromosomes of the different types is, of course, only half as great in the second spermatocyte, as it is in the first spermatocyte, and therefore there is not such certainty in identifying the various individuals of the different types. But the same size ratio seems to exist and the chromosomes of the different types can readily be arranged in a graded series as regards size, just as in the first spermatocyte.

It has been shown by this study that the chromosomes of *Scolopendra heros* cannot be considered as ephemeral structures, which have one appearance in one cell and present an entirely different form in another cell of similar history. Any study except a very superficial one must lead to an entirely different conclusion. By a study of many hundreds of cells in various stages of mitosis it has been found that the number of chromosomes in the primary spermatocytes is absolutely constant and invariable. Furthermore, these chromosomes show other characteristics, which speak very strongly for their individuality. The ordinary chromosomes are divisible into three types on the basis of the shape they assume in the prophase and metaphase of the first maturation division, and in their relation to the mantle fibers of the spindle. The individuals of each type of structure are invariably of the same number and in all favorable cases each chromosome of a given type is distinguishable from the others of a similar shape by a difference in size.

In addition, several of the chromosomes possess certain individual peculiarities aside from shape and size, which serve

further to characterize them. One of the cross-shaped tetrads is tardy in orienting itself in the plane of the spindle. Another chromosome, one of the rod-shaped ones, shows a much more striking and fundamental peculiarity, in that it differs from all of the rest of the bivalent chromosomes in the plane of its divisions in the first and second spermatocytic mitoses. The accessory chromosome shows still more striking peculiarities, differing from the others in its origin, valence, behavior in the prophase, relation to the mantle fibers of the spindle and in its distribution to but one half of the resulting cells.

All of the facts enumerated above offer evidence which seems conclusive that the chromosomes of *Scolopendra heros*, during the spermatocyte stages at least, must be considered as distinct entities, each one possessing certain well defined peculiarities which are as characteristic for any given chromosome of the spermatocytes as are the peculiarities of a species of animals. I believe that eventually in many animals it will be possible to make this statement still broader and to demonstrate the continuity of the individual elements from cell generation to cell generation. We will, then, be able to say that, while in different cell generations or different conditions of cell activity, the appearance and behavior of any given chromosome may be quite different, just as is true of many animals in different stages of their existence, yet in a similar cell generation any particular chromosome will present the same appearance and will behave in the same manner. I believe that the condition described above is true in *Scolopendra heros* but several facts conspire to make it impossible of physical demonstration.

These difficulties are mechanical difficulties and have to do with the small size of the chromosomes in the spermatogonial stages and the close aggregation of these elements in the karyosphere of the growth period. The difficulties due to the small size of the chromosomes in the spermatogonial stages appears insurmountable, and the only evidences of individuality which they present have to do with their absolute constancy in number, and with the very characteristic behavior of the accessory—it being the only element which can be identified at all stages. We might reason from this that because one of the elements

displays unmistakable individuality all of the chromosomes possess individuality. This argument has been made in other cases and, while the continuity of the accessory chromosome *does* offer valuable evidence in support of the individual continuity of the chromosomes in general, it cannot be said to establish the truth of the general theory.

The difficulty of establishing the individuality of the chromosomes during the growth period would seem fully as great as during the spermatogonial period. During all the stages in which the karyosphere exists the chromosomes are so densely aggregated that it is impossible to distinguish the separate elements. But even at this time it is possible in favorable cases to distinguish the accessory chromosomes and to discern the outlines of some of the other elements. Furthermore, as I have shown in previous papers (Blackman, :05, *op. cit.*) the chromosomes enter the karyosphere as distinct bivalent elements, and at the end of the growth period arise from it as distinct chromatic segments of the same number and character as in the earlier stage.

The chromatin segments entering the karyosphere are bivalent threads formed by the union and subsequent diffusion of two spermatogonial chromosomes. The point of union of synapsis shows very plainly as a distinct interruption of the chromatin granules near the middle of the segment, the interval being bridged by linin fibers. In favorable sections of the karyosphere (*i. e.*, those in which the stain has been sufficiently extracted) it is seen that this body is made up of a number of chromatin segments closely massed about the accessory. The chromosomes on leaving the karyosphere are of the same structure as when they entered, are of the same number and in appearance differ from those of an earlier period in size only. In fact, the larger spermatocyte chromosomes possess nearly as great a bulk as the entire chromosome group of the spermatogonium, this immense increase in size being accompanied by a growth of other parts of the cell, which is proportionally even greater.

It would appear then, that during certain stages of the spermatogenesis of *Scolopendra* it is possible to demonstrate absolutely that each chromosome is a distinct unit characterized by certain definite and constant peculiarities and that the continuity of

each element can be traced from the early prophase of the first spermatocyte to the anaphase of the second maturation division. In other words, it is evident that during this very important period of their history the chromosomes show complete individuality. In other stages, namely, in the spermatogonia and during the growth period, it cannot be claimed that the continuity of the chromosomes is actually demonstrated in *Scolopendra*, although evidence strongly supporting such a view undoubtedly exists.

SUMMARY.

The chromosome group of the primary spermatocytes of *Scolopendra heros* is made up of sixteen bivalent chromosomes (tetrads) and one univalent chromosome (dyad), the accessory chromosome.

The chromosomes show such constancy in shape in the prophase and metaphase of the primary spermatocytes, and in their relation to the mantle fibers of the first maturation spindle, that they seem naturally to group themselves under four distinct types. These may be designated respectively, as the cross-shaped tetrads, the double-V-shaped tetrads, the rod-shaped tetrads, and a single-rod-shaped dyad.

The cross-shaped tetrads are six in number and may be arranged in a graded series as regards size, the difference in bulk being sufficiently great to allow the individual chromosomes of this type to be distinguished. One of the chromosomes of this type (the largest one) can furthermore often be identified by its tendency to lag behind the others during the early metaphase.

Five of the tetrads are of the double-V shape. The individuals of this type also may be distinguished by differences in bulk.

The rod-shaped tetrads are present to the number of five. These show constant size relations and may readily be arranged in a graded series as regards magnitude. One of the tetrads of this type differs from the others in the form it assumes during actual division. It seems to divide transversely, while the others are dividing longitudinally.

The accessory chromosome is univalent and passes to one of the secondary spermatocytes without division. During the

metaphase it is connected by mantle fibers to only one pole of the spindle.

As a result of the first spermatocyte mitosis fifteen of the tetrads are divided longitudinally (equationally), while the one remaining tetrad divides transversely (reductionally). The failure of the accessory chromosome to divide is, also, in effect a reductional division.

During the later stages of the first maturation division and during the metaphase of the second spermatocyte, it is possible to distinguish the daughter chromosomes derived from the several types of tetrads, by their shape and their relations to the mantle fibers. The individuals of the various types show the same size ratio as exists between the chromosomes of the first spermatocyte, although, of course, the actual difference in bulk is but half as great.

The above results seem to establish as a fact, or at least as a very strong probability, that the chromosomes of *Scolopendra heros* are distinct and definite individuals, which, under similar circumstances, *i. e.*, in the same cell generation, show a remarkable constancy in form, relative size, and in their attachment to the mantle fibers. This constancy of form, size and behavior, affords a strong argument in favor of the theory of the individuality of the chromosomes in this species in particular and adds support to the evidence derived from the study of other forms, to the general application of the theory.

LABORATORY OF ZOÖLOGY,
SYRACUSE UNIVERSITY,
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Explanation of Plate I.

All drawings were made by the author with the aid of a camera lucida. The optical equipment consisted of a Zeiss apochromatic objective of 2 mm. focus and a number 12 compensating ocular, the source of the light being a Welsbach mantle The original magnification was 2,300 diameters and the drawings were reduced one fifth in reproduction, making the final magnification 1,840 diameters.

The seventeen chromosomes arranged in each horizontal row represent the chromosomes of a single cell, as seen in a side view of the spindle in the metaphase. They are arranged as follows: the six which show the characteristic cruciform shape comprise the first six of each row and are lettered A, B, C, D, E and F. Those showing the double-V shape—five in number—are lettered G, H, I, J and K. Those corresponding to the double-rod type of structure are lettered L, M, N, O and P. The seventeenth and last chromosome in each row is the accessory and is distinguished by the letter Q. The individuals of each type of structure are further arranged in a graded series as regards size, the largest first, etc.

Figs. 1–15 represent the chromosome groups in the metaphase of the large type of first spermatocytes.


A B C D E F G H I J K L M N O P Q
1
2
3
4
5
6
7
8
9
10

Explanation of Plate II.

Figs. 16–18 represent the chromosomes of the small type of spermatocyte in a similar stage.

Figs. 19 and 20 represent the chromosomes of the two second spermatocytes, derived from one primary spermatocyte. The individual chromosomes are arranged so as to correspond in position to the parent chromosomes as seen in the other figures. As will be seen, in one cell the accessory chromosome is not present, it being distributed to only half of the secondary spermatocytes.

M. W. BLACKMAN